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[Designation of Document] specification

[Title of the Invention] SOLID SUPPORT HAVING ELECTROSTATIC LAYER AND APPLICATION THEREOF

[Claims]

[Claim 1]

A solid support having an electrostatic layer for electrostatically attracting a nucleic acid molecule and a functional group capable of covalently binding to a nucleic acid molecule on a substrate.

[Claim 2]

The solid support according to claim 1, wherein the surface of the substrate is surface-treated with at least one kind selected from a diamond, a soft diamond, a carbonaceous matter and carbide.

[Claim 3]

The solid support according to claim 1 or 2, wherein the electrostatic layer comprises an amino group-containing compound which does not covalently bind to the substrate.

[Claim 4]

The solid support according to claim 1 or 2, wherein the electrostatic layer is composed of an amino group-containing compound which covalently binds to the substrate and the amino group-containing compound has an amino group at the terminus to which the substrate does not bind.

[Claim 5]

The solid support according to any one of claims 1 to 3, which is obtained by depositing a compound having an unsubstituted or monosubstituted amino group and a carbon compound on the substrate and then introducing a functional group capable of covalently binding to a nucleic acid molecule.

[Claim 6]

The solid support according to any one of claims 1 to 4, which is obtained by dipping the substrate in a solution containing a compound having an unsubstituted or monosubstituted amino group and then introducing a functional group capable of covalently binding to a nucleic acid molecule.

[Claim 7]

The solid support according to claim 6, wherein the compound having an unsubstituted or monosubstituted amino group is polyarylamine.

[Claim 8]

The solid support according to any one of claims 1 to 7, wherein the nucleic acid molecule is DNA.

[Claim 9]

An immobilized nucleic acid molecule, which comprises a nucleic acid molecule immobilized on the solid support according to any one of claims 1 to 8.

[Claim 10]

A method of producing a solid support characterized by depositing a compound having an unsubstituted or monosubstituted amino group and a carbon compound on the substrate and then introducing a functional group capable of covalently binding

to a nucleic acid molecule.

[Claim 11]

A method of producing a solid support characterized by dipping the substrate in a solution containing a compound having an unsubstituted or monosubstituted amino group and then introducing a functional group capable of covalently binding to a nucleic acid molecule.

[Detailed Explanation of the Invention]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to a support for immobilizing DNA or the like and an immobilized nucleic acid molecule.

[0002]

[Technical Background of the Invention]

Conventionally, in a DNA amplification reaction or the like, to obtain a predetermined quantity of a target DNA, it is necessary to repeat the heat cycle, which consists of 1) raising the temperature of a sample to 95C° in order to disrupt the hydrogen bond of the double-stranded DNA, 2) subsequently, lowering the temperature of the sample to 45C° in order to recombine the DNA to a primer for replication, 3) further, raising the temperature of the sample to 74C° in order to replicate the DNA by extending the primer with a heat-resistant polymerase, a number of times. In such a DNA amplification reaction, the foregoing heat cycle was carried out by putting a sample into a container made of a synthetic resin or the like, and accommodating this container into an aluminum block.

However, the foregoing heat cycle consumed a lot of time, and it took several hours to obtain a required amount of DNA. In addition, there also has been a drawback that the accuracy of the temperature control is low and hence, DNA other than the target may be replicated in some cases.

[0003]

In view of the foregoing problems, as a support capable of easily immobilizing DNA and suitable for replicating DNA by DNA amplification reaction, a solid support which comprises, on the surface of a substrate, a surface-treated layer and chemically modified layer having a functional group capable of covalently binding to a nucleic acid molecule sequentially has been developed (for example, WO 00/22108, WO 02/12891 or JP-A-2002-82116).

However, the amount of DNA immobilized on the foregoing solid support to DNA is not always sufficient, therefore, the emergence of a solid support capable of immobilizing DNA in a higher proportion has been awaited.

[0004]

[Problem to be solved by the Invention]

The object of the present invention is to provide a solid support capable of immobilizing nucleic acid molecules in a higher proportion.

[0005]

[Means for solving the Problem]

The present inventors carried out intensive studies in order to solve the foregoing problems, and as a result, found out that the immobilized amount of nucleic acid molecules is significantly improved by further providing an electrostatic layer for electrostatically attracting nucleic acid molecules on a solid support having a functional group capable of covalently binding to a nucleic acid molecule on a substrate, thereby accomplishing the present invention.

[0006]

That is, the present invention includes the following inventions.

(1) A solid support having an electrostatic layer for electrostatically attracting a nucleic acid molecule and a functional group capable of covalently binding to a nucleic acid molecule on a substrate.

(2) The solid support according to the foregoing (1), in which the surface of the substrate is surface-treated with at least one kind selected from diamond, a soft diamond, a carbonaceous matter and carbide.

(3) The solid support according to the foregoing (1) or (2), in which the electrostatic layer comprises an amino group-containing compound which does not covalently bind to the substrate.

[0007]

(4) The solid support according to the foregoing (1) or (2), in which the electrostatic layer is composed of an amino group-containing compound which covalently binds to the substrate and the amino group-containing compound has an amino group at the terminus to which the substrate does not bind.

(5) The solid support according to any one of the foregoing (1) to (3), which is obtained by depositing a compound having an unsubstituted or monosubstituted amino group and a carbon compound on the substrate and then introducing a functional group capable of covalently binding to a nucleic acid molecule.

(6) The solid support according to any one of the foregoing

(1) to (4), which is obtained by dipping the substrate in a solution containing a compound having an unsubstituted or monosubstituted amino group and then introducing a functional group capable of covalently binding to a nucleic acid molecule.

[0008]

(7) The solid support according to the foregoing (6), in which the compound having an unsubstituted or monosubstituted amino group is polyarylamine.

(8) The solid support according to any one of the foregoing (1) to (7), in which the nucleic acid molecule is DNA.

(9) An immobilized nucleic acid molecule, which comprises a nucleic acid molecule immobilized on the solid support according to any one of the foregoing (1) to (8).

[0009]

(10) A method of producing a solid support characterized by depositing a compound having an unsubstituted or monosubstituted amino group and a carbon compound on the substrate and then introducing a functional group capable of covalently binding to a nucleic acid molecule.

(11) A method of producing a solid support characterized by dipping the substrate in a solution containing a compound having an unsubstituted or monosubstituted amino group and then introducing a functional group capable of covalently binding to a nucleic acid molecule.

[0010]

[Mode for carrying out the Invention]

Examples of a material of the substrate to be used in

the present invention include, for example, silicone, glass, fiber, wood, paper, ceramics, andplastic (e.g., polyesterresin, polyethylene resin, polypropylene resin, ABS resin (acrylonitrile butadiene styrene resin), nylon, acrylic resin, fluorocarbon resin, polycarbonate resin, polyurethane resin, methylpentene resin, phenolic resin, melamine resin, epoxy resin, polyvinyl chloride resin).

[0011]

In the case where the foregoing members are used as the material of the substrate, it is more preferred that surface treatment be carried out in order to firmly immobilize a compound for introducing a functional group capable of covalently binding to a nucleic acid molecule on the substrate.

[0012]

For the surface treatment, it is preferred to use any of synthetic diamond, high-pressure synthetic diamond, natural diamond, a soft diamond (e.g., a diamond-like carbon), amorphous carbon, a carbonaceous matter (e.g., graphite, fullerene or carbon nanotube), a mixture thereof, or a laminated product thereof. In addition, carbide such as hafnium carbide, niobium carbide, silicon carbide, tantalum carbide, thorium carbide, titanium carbide, uranium carbide, tungsten carbide, zirconium carbide, molybdenum carbide, chromium carbide or vanadium carbide may be used. The term of soft diamond here is used as a collective term of a partial diamond structure, which is a mixture of diamond and carbon, such as, so-called a diamond-like carbon (DLC), and the mixing ratio thereof is not particularly

limited.

[0013]

As one example of the surface-treated substrate, a substrate in which a film has been formed with a soft diamond on a slide glass is exemplified. It is preferred that such a substrate be produced by the ionization deposition method in a mixed gas containing 0 to 99% by volume of hydrogen gas and the balance of methane gas (100 to 1% by volume) with a diamond-like carbon.

It is preferred that the thickness of the surface-treated layer be 1 nm to 100 μm.

[0014]

The formation of the surface-treated layer on the substrate can be carried out by a known method such as the microwave plasma CVD (chemical vapor deposition) method, ECRCVD (electric cyclotron resonance chemical vapor deposition) method, IPC (inductive coupled plasma) method, DC sputtering method, ECR (electric cyclotron resonance) sputtering method, ion plating method, arc ion plating method, EB (electron beam) deposition method, resistance heating vapor deposition method, ionization deposition method, arc deposition method, laser deposition method.

[0015]

Examples of the substrate to be used in the present invention include, not only the structure in which the surface-treated layer has been formed as described above, but also synthetic diamond, high-pressure synthetic diamond,

natural diamond, a soft diamond (e.g., a diamond-like carbon), amorphous carbon; metals such as gold, silver, copper, aluminum, tungsten and molybdenum; plastic (such as polyester resin, polyethylene resin, polypropylene resin, ABS resin, nylon, acrylic resin, fluorocarbon resin, polycarbonate resin, polyurethane resin, methylpentene resin, phenolic resin, melamine resin, epoxy resin, polyvinyl chloride resin); the one formed by mixing and combining powder of the foregoing metal, powder of ceramic or the like with the foregoing resin as a binder; the one obtained by sintering at a high temperature a material such as powder of the foregoing metal or powder of ceramic, which has been powder-pressed with press-molding machine. In addition, the substrate may be a laminated product or a composite of the foregoing materials (for example, a composite of diamond and another substance, (e.g. a two-phase substance)).

[0016]

The shape and the size of the substrate are not particularly limited. However, with regard to the shape, it may be in the form of plate, thread, sphere, polygon, powder and the like, and with regard to the size, in the case of using the one in the form of plate, it is generally about 0.1 to 100 mm of width, 0.1 to 100 mm of length and 0.01 to 10 mm of thickness.

[0017]

In addition, on the front face or back face of the substrate, a monolayer of Ti, Au, Pt, Nb, Cr, TiC, TiN or the like, or a composite layer thereof may be formed as a reflective layer. The thickness of the reflective layer is preferably 10 nm or

more, more preferably 100 nm or more, because it is necessary to be uniform throughout the surface.

[0018]

In the case of using glass as the substrate, it is also preferred that the surface be intentionally roughened within the range of 1 nm to 1000 nm expressed in Ra (JIS B 0601). Such roughened surface is advantageous in that the surface area of the substrate is increased, whereby a large amount of DNA probes or the like can be immobilized at a high density.

[0019]

The solid support of the present invention is provided with an electrostatic layer for electrostatically attracting a nucleic acid molecule.

The electrostatic layer is not particularly limited as long as it attracts a nucleic acid molecule electrostatically and improves the immobilized amount of nucleic acid molecules, however, it can be formed by, for example, using a positively charged compound such as an amino group-containing compound.

[0020]

Examples of the foregoing amino group-containing compound include a compound having an unsubstituted amino group ($-NH^2$) or an amino group, which has been monosubstituted with an alkyl group having 1 to 6 carbon atoms or the like (-NHR; R is a substituent), and for example, ethylenediamine, hexamethylenediamine, n-propylamine, monomethylamine, dimethylamine, monoethylamine, diethylamine, arylamine, aminoazobenzene, aminoalcohol, (e.g., ethanolamine), acrinol,

aminobenzoic acid, aminoanthraquinone, amino acids (glycine, alanine, valine, leucine, serine, threonine, cysteine, methionine, phenylalanine, tryptophan, tyrosine, proline, cystine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine and histidine), aniline, a polymer thereof (e.g., polyarylamine and polylysine) and a copolymer thereof; a polyamine (polyvalent amine) such as 4,4',4''-triaminotriphenylmethane, triamterene, spermidine, spermin and putrescine.

The electrostatic layer may be formed without covalently binding to the substrate or the surface-treated layer, or may be formed by covalently binding to the substrate or the surface-treated layer.

[0021]

In the case of forming the electrostatic layer without covalently binding to the substrate or the surface-treated layer, a carbonaceous film containing an amino group is formed by, for example, introducing the foregoing amino group-containing compound into a film-forming apparatus when the surface-treated layer is formed. In addition, the surface-treated layer may be a multiple layer in which a film containing an amino group is formed after forming an adherent layer.

[0022]

Further, in the case of forming the electrostatic layer without covalently binding to the substrate or the surface-treated layer, it is preferred to introduce a functional group capable of covalently binding to a nucleic acid molecule

after depositing the forgoing compound having an unsubstituted or monosubstituted amino group and a carbon compound on the substrate in order to enhance the affinity, namely adhesiveness between the electrostatic layer and the substrate or the surface-treated layer. The carbon compound to be used here is not particularly limited as long as it can be supplied as a gas, however, preferred are, for example, methane, ethane and propane that are gas at a normal temperature. As the method for deposition, the ionization deposition method is preferred. As the condition of the ionization deposition method, it is preferred that the working pressure be in the range of 0.1 to 50 Pa and the accelerating voltage be in the range of 200 to 1000 V.

[0023]

In the case of forming the electrostatic layer by covalently binding to the substrate or the surface-treated layer, the electrostatic layer can be formed by, for example, irradiating the substrate or the substrate provided with the surface-treated layer with ultraviolet rays in chlorine gas to chlorinate the surface, and reacting, among the foregoing amino group-containing compounds, for example, a polyvalent amine such as polyarylamine, polylysine 4,4',4''-triaminotriphenyl-methane or triamterene to introduce an amino group into the terminus to which the substrate does not bind.

[0024]

Further, in the case of carrying out the reaction of introducing a functional group capable of covalently binding

to a nucleic acid molecule into the substrate provided with the electrostatic layer (e.g., introduction of a carboxyl group by using a dicarboxylic acid or polyvalent carboxylic acid) in a solution, it is preferred to introduce a functional group capable of covalently binding to a nucleic acid molecule after dipping the substrate in a solution containing the foregoing compound having an unsubstituted or monosubstituted amino group. Examples of the solvent for the foregoing solution include, for example, water, N-methylpyrrolidone and ethanol.

[0025]

In the case of introducing a carboxyl group by using a dicarboxylic acid or polyvalent carboxylic acid into the substrate provided with the electrostatic layer, it is preferred to activate it with N-hydroxysuccinimide and/or a carbodiimide in advance, or to carry out the reaction in the presence of N-hydroxysuccinimide and/or a carbodiimide.

[0026]

In the case of forming the electrostatic layer by dipping the substrate in a solution containing the compound having an unsubstituted or monosubstituted amino group, if polyarylamine is used as the amino group-containing compound, the adhesiveness to the substrate will be excellent and the immobilized amount of the nucleic acid molecule will be more improved.

It is preferred that the thickness of the electrostatic layer be in the range of 1 nm to 500 μm.

[0027]

As described above, the surface of the substrate provided

with the electrostatic layer is chemically modified in order to introduce a functional group capable of covalently binding to a nucleic acid molecule.

Examples of the foregoing functional group include, for example, a carboxyl group, active ester group, haloformyl group, hydroxyl group, sulfate group, cyano group, nitro group, thiol group and amino group.

[0028]

Examples of the compound to be used for introducing a carboxyl group as the functional group include, for example, a halocarboxylic acid represented by the formula: $X-R^1-COOH$ (wherein X represents a halogen atom, and R^1 represents a divalent hydrocarbon group having 1 to 12 carbon atoms) such as chloroacetic acid, fluoroacetic acid, bromoacetic acid, iodoacetic acid, 2-chloropropionic acid, 3-chloropropionic acid, 3-chloroacrylic acid or 4-chlorobenzoic acid; a dicarboxylic acid represented by the formula: $HOOC-R^2-COOH$ (wherein R^2 represents a single bond or a divalent hydrocarbon group having 1 to 12 carbon atoms) such as oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid or phthalic acid; a polyvalent carboxylic acid such as polyacrylic acid, polymethacrylic acid, trimellitic acid or butanetetracarboxylic acid; a keto acid or aldehyde acid represented by the formula: R^3-CO-R^4-COOH (wherein R^3 represents a hydrogen atom or a divalent hydrocarbon group having 1 to 12 carbon atoms and R^4 represents a divalent hydrocarbon group having 1 to 12 carbon atoms); a monohalide of dicarboxylic acid

represented by the formula: X-OC-R^5-COOH (wherein X represents a halogen atom and R^5 represents a single bond or a divalent hydrocarbon group having 1 to 12 carbon atoms) such as monochloride succinate or monochloride malonate; an acid anhydride such as phthalic acid anhydride, succinic acid anhydride, oxalic acid anhydride, maleic acid anhydride or butanetetracarboxylic acid anhydride.

[0029]

With regard to the carboxyl group introduced as described above, active esterification thereof can be carried out with a dehydration condensing agent such as cyanamide or carbodiimide (e.g. 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide) and a compound such as N-hydroxysuccinimide.

[0030]

Examples of the compound to be used for introducing a haloformyl group as the functional group include, for example, a dihalide of dicarboxylic acid represented by the formula: X-OC-R^6-CO-X (wherein X represents a halogen atom, and R^6 represents a single bond or a divalent hydrocarbon group having 1 to 12 carbon atoms) such as chloride succinate or chloride malonate.

Examples of the compound to be used for introducing a hydroxyl group as the functional group include, for example, a hydroxy acid or phenol acid represented by the formula: HO-R^7-COOH (wherein R^7 represents a divalent hydrocarbon group having 1 to 12 carbon atoms).

Examples of the compound to be used for introducing an

amino group as the functional group include, for example, an amino acid.

[0031]

The foregoing compound forms an amide bond by condensing the carboxyl group with the amino group in the electrostatic layer.

Among the foregoing compounds, the polyvalent carboxylic acid such as polyacrylic acid, polymethacrylic acid, trimellitic acid or butanetetracarboxylic acid can be also used for improving hydrophilicity.

[0032]

On the support of the present invention, either of the nucleic acid molecules, DNA and RNA, can be immobilized. The number of bases in DNA or RNA is generally 1 to 200, preferably 5 to 150. In addition, in the case of DNA, either a single-stranded or double-stranded chain can be immobilized.

The support of the present invention can be used in DNA amplification reaction, for example. Further, by immobilizing the terminal base of an oligonucleotide on a terminal hydroxyl group or terminal carboxyl group through a hydrogen bond with the use of the support of the present invention, and further immobilizing DNA having a base sequence complementary to this oligonucleotide, the resultant product can be used as a DNA library chip. In addition, by immobilizing a nucleotide, oligonucleotide, DNA fragment or the like instead of DNA, the resultant product can be used as a library.

[0033]

[Examples]

Hereunder, the present invention will be explained with reference to the Examples, however, the present invention is not intended to be limited thereto.

(Embodiment 1)

Introduction of Amino Group-containing Compound into Chamber when Applying Surface-treated Layer to Substrate (1)

DLC layer was formed at a thickness of 10 nm on a slide glass of 25 mm (width)×75 mm (length)×1 mm (thickness) by the ionization deposition method, at an accelerating voltage of 0.5 kv, using a mixed gas of 95% by volume of methane gas and 5% by volume of hydrogen gas as material. Then, using methane gas as a carrier gas, at the rate of 5 cm^3/minute, it was introduced into a chamber through the ethylenediamine incubated at 15C°. At a working pressure of 2 Pa and an accelerating voltage of 0.5 kv, using methane and ethylenediamine as material, a layer consisting of C, N and H was formed at a thickness of 10 nm.

[0034]

Then, after butanetetracarboxylic acid anhydride, as a polyvalent carboxylic acid, was condensed with the amino group in the surface-treated layer consisting of C, N and H formed with methane and ethylenediamine as material, it was activated by being dipped in an activation solution, in which 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide and 20 mM N-hydroxysuccineimide had been dissolved in 300 ml of 0.1 M phosphate buffer (pH 6), for 30 minutes.

[0035]

Then, about 1 nl of 500 bp Cy3-labeled double-stranded DNA, which had been amplified by PCR using λDNA prepared at a concentration of 0.1μg/μl as a template, was spotted on the substrate using a microarray maker. Then, after it was heated in an oven at 80C° for 3 hours and washed with 2×SSC/0.2% SDS, the intensity of fluorescence of the spotted DNA was measured.

As a result, the intensity of fluorescence was 36,050. Further, when the intensity of fluorescence was measured after performing washing with 2×SSC/0.2% SDS at 95C°, it was 35,540, which was hardly decreased at all.

[0036]

As a comparison, after a slide glass was dipped in an ethanol solution containing 2% by weight of 3-aminoprolyltriethoxysilane for 10 minutes, it was taken out, washed with ethanol, and dried at 110C° for 10 minutes. Then, after succinic acid anhydride was condensed with this substrate to which an amino group was introduced, it was activated by being dipped in an activation solution, in which 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide and 20 mM N-hydroxysuccineimide had been dissolved in 300 ml of 0.1 M phosphate buffer (pH6), for 30 minutes. After 500 bp Cy3-labeled double-stranded DNA, which had been amplified by PCR using λDNA as a template, was immobilized in the same manner on the thus obtained substrate, the substrate was washed with 2×SSC/0.2% SDS. As a result, the intensity of fluorescence was 23,500. Further, when the intensity of fluorescence was measured after performing washing with 2×SSC/0.2% SDS at 95C°, it was 23,000,

which was hardly decreased at all.

In other word, by using a covalent bond type substrate that hardly has an electrostatic layer, although DNA can be immobilized more firmly by a covalent bond, the intensity of a fluorescence signal was not increased.

[0037]

Further, after 500 bp Cy3-labeled double-stranded DNA, which had been amplified by PCR using λDNA as a template, was immobilized in the same manner on a substrate that does not have an electrostatic layer (after 5% polyacrylic acid aqueous solution was applied on a slide glass and dried, ultraviolet rays were irradiated for 60 minutes to make it insoluble. Then, it was activated by being dipped in an activation solution, in which 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide and 20 mM N-hydroxysuccineimide had been dissolved in 300 ml of 0.1 M phosphate buffer (pH 6), for 30 minutes), the substrate was washed with 2×SSC/0.2% SDS. As a result, though the polyacrylic acid film was peeled off, the intensity of fluorescence was 26,220 in the region where the layer remained. Further, the polyacrylic acid film was completely peeled off when being washed with 2×SSC/0.2% SDS at 95C°.

[0038]

(Embodiment 2)

Introduction of Amino Group-containing Compound into Chamber when Applying Surface-treated Layer to Substrate (2)

To a slide glass of 25 mm (width) ×75 mm (length) ×1 mm (thickness) by the ionization deposition method, using methane

gas as a carrier gas, at the rate of 5 cm^3/minute, it was introduced into a chamber through the ethylenediamine incubated at 15C°. At a working pressure of 2 Pa and an accelerating voltage of 0.5 kv, using methane and ethylenediamine as material, a layer consisting of C, N and H was formed at a thickness of 20 nm.

[0039]

Then, after polyacrylic acid, as a polyvalent carboxylic acid, was condensed with the amino group in the surface-treated layer consisting of methane and ethylenediamine in the presence of 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, it was activated by being dipped in an activation solution, in which 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide and 20 mM N-hydroxysuccineimide had been dissolved in 300 ml of 0.1 M phosphate buffer (pH 6), for 30 minutes.

[0040]

Then, about 1 nl of 500 bp Cy3-labeled double-stranded DNA, which had been amplified by PCR using λDNA prepared at a concentration of 0.1μg/μl as a template, was spotted on the substrate using a microarray maker. Then, after it was heated in an oven at 80C° for 3 hours and washed with 2×SSC/0.2% SDS, the intensity of fluorescence of the spotted DNA was measured.

As a result, the intensity of fluorescence was 34,050. Further, when the intensity of fluorescence was measured after performing washing with 2×SSC/0.2% SDS at 95C°, it was 33,500, which was hardly decreased at all.

[0041]

As a comparison, after 500 bp Cy3-labeled double-stranded

DNA, which had been amplified by PCR using λDNA as a template, was immobilized in the same manner on a commercially available electrostatic type substrate (manufactured by Matsunami Glass Ind., LTD.; a substrate which is a slide glass applied with aminosilane (a silane coupling agent)), the substrate was washed with 2×SSC/0.2% SDS. As a result, the intensity of fluorescence was 35,460. Further, when the intensity of fluorescence was measured after performing washing with 2×SSC/0.2% SDS at 95C°, it was decreased to 26,210.

[0042]

Further, after 500 bp Cy3-labeled double-stranded DNA, which had been amplified by PCR using λDNA as a template, was immobilized in the same manner on a substrate that does not have an electrostatic layer (after 5% polyacrylic acid aqueous solution was applied on a slide glass and dried, ultraviolet rays were irradiated for 60 minutes to make it insoluble. Then, it was activated by being dipped in an activation solution, in which 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide and 20 mM N-hydroxysuccineimide had been dissolved in 300 ml of 0.1 M phosphate buffer (pH 6), for 30 minutes), the substrate was washed with 2×SSC/0.2% SDS. As a result, though the polyacrylic acid film was peeled off, the intensity of fluorescence was 26,220 in the region where the layer remained. Further, the polyacrylic acid film was completely peeled off when being washed with 2×SSC/0.2% SDS at 95C°.

[0043]

(Embodiment 3)

Formation of Electrostatic Layer by Post-treatment

DLC layer was formed at a thickness of 10 nm on a slide glass of 25 mm (width)×75 mm (length)×1 mm (thickness) by the ionization deposition method, at an accelerating voltage of 0.5 kv, using a mixed gas of 95% by volume of methane gas and 5% by volume of hydrogen gas as material.

[0044]

Then, it was chlorinated by being irradiated with ultraviolet rays for 30 minutes in chlorine gas. Then, the substrate was dipped in a polyacrylic amine aqueous solution (0.1 g/l), whereby an electrostatic layer was formed.

Then, after polyacrylic acid, as a polyvalent carboxylic acid, was condensed with the amino group in the electrostatic layer in the presence of 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide, it was activated by being dipped in an activation solution, in which 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide and 20 mM N-hydroxysuccineimide had been dissolved in 300 ml of 0.1 M phosphate buffer (pH 6), for 30 minutes.

[0045]

Then, about 1 nl of 500 bp Cy3-labeled double-stranded DNA, which had been amplified by PCR using λDNA prepared at a concentration of 0.1μg/μl as a template, was spotted on the substrate using a microarray maker. Then, after it was heated in an oven at 80C° for 3 hours and washed with 2×SSC/0.2% SDS, the intensity of fluorescence of the spotted DNA was measured.

[0046]

As a result, the intensity of fluorescence was 35,000. Further, when the intensity of fluorescence was measured after performing washing with 2×SSC/0.2% SDS at 95C°, it was 34,500, which was hardly decreased at all.

[0047]

As a comparison, after 5% polyacrylic acid aqueous solution was applied to a slide glass on which DLC was formed at a thickness of 10 nm and dried, ultraviolet rays were irradiated for 60 minutes to make it insoluble. Then, it was activated by being dipped in an activation solution, in which 0.1 M 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide and 20 mM N-hydroxysuccineimide had been dissolved in 300 ml of 0.1 M phosphate buffer (pH 6), for 30 minutes. After 500 bp Cy3-labeled double-stranded DNA, which had been amplified by PCR using λDNA as a template was immobilized in the same manner, and washed with 2×SSC/0.2% SDS, the polyacrylic acid film was completely peeled off.

[0048]

[Advantageous effect]

On the solid support of the present invention, a larger amount of nucleic acid molecules can be immobilized than on the conventional solid support, and it can be immobilized firmly through a covalent bond. Therefore, the detection sensitivity and reliability that were the problems of the conventional DNA array can be improved. Accordingly, it is possible to aim at generalizing a DNA array.

[Brief Description of the Drawings]

[Fig. 1]

A schematic view showing a solid support of the present invention and one example of a conventional product.

[Designation of Document] Abstract

[Abstract]

[Task]

To provide a solid support capable of immobilizing nucleic acid molecules in a high proportion.

[Means for solving the task]

The solid support comprises a substrate and, provided thereon, an electrostatic layer for electrostatically attracting nucleic acid molecules and functional groups capable of covalent bond with nucleic acid molecules.

[Selected drawing] Fig. 1